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Impact of Lamda-Cyhalothrin on the Biochemical Parameters of *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Widespread applications of pesticides such as Lambda cyhalothrin to boost crops production have frequently led to contamination of the fresh water ecosystem in Nigeria.
Materials and Methods: In this study, *Clarias gariepinus* were exposed to sub-lethal concentrations of Lambda-cyhalothrin pesticide. The 96 h LC_{50} of lambda cyhalothrin to the fish was estimated at 3.98mgl^{-1}. Mortality of 100% and 10% were observed in fish exposed to 12.00 mg l^{-1} and 1.25 mg l^{-1} of Lambda cyhalothrin respectively as compared to no mortality recorded in the control group. Varying degrees of abnormal behaviours like air gulping, hyperactivity, erratic movement, skin discoloration and jerky movements were observed during the 96 hrs exposure period of the fish to Lambda compared to the control. Exposure to sub-lethal concentrations of Lambda cyhalothrin at 0, 0.25, 0.50 and 1.00 mgl^{-1} and for 15 days at 5 days intervals that is 5, 10 and 15days led to changes in the biochemical parameters.
Results: In the Biochemical parameters analysed, there was significant (P<0.5) increase in the

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mean values of Albumin, Aspartate Amino Transfarase (AST), Alanine Amino Transaferase (ALT), Alkaline Phosphate (ALP) and Creatinine compares to the control group. There was no significant difference ($p>0.05$) between the values of water quality assays in control and treated group.
Conclusion: The biomarkers measured could be useful tools for monitoring effects of other pesticides on aquatic organisms. However, further studies could be done to investigate their mode of action to strike a balance between protection of aquatic biota and discharges of these pesticides and their metabolites to aquatic environments.

Keywords: Impact; biochemical parameters; Lambda; pesticide.

1. INTRODUCTION

"In order to increase food production due to the increasing human population, different types of pesticides are applied to meet up the demand for food production through agricultural practices" (Nwani, 2015). "Unfortunately, the indiscriminate and uncontrolled applications of these pesticides in agricultural land have resulted to ecotoxicological effects to the aquatic biota when it gets washed into aquatic ecosystem through erosion. Aquatic environment is particularly one vulnerable area as it is the ultimate recipient of pollutants due to basin drainage. The aquatic ecosystems have been known to receive a wide spectrum of pollutants, which may be introduced to them directly or indirectly" (Oyibo, et al., 2014). "The use of pesticides has continued to increase as it is still considered the most effective method to reduce pests and increase crop growth in agriculture" (Gupta, 2008). "The indiscriminate use of pesticides has resulted in large scale reduction in aquatic productivity. Pesticides have different diverse impacts on aquatic animals especially fishes which are of economic importance and high value from the point of biological conservation" [1]. "Environmental pollution by pesticides has become a serious problem in terms of global conservation and animal and human health" [2,3].

"Lambda-cyhalothrin (LCT) is a synthetic pyrethroid that has immediate and persistent effects activity against a large variety of arthropods, and also harmful both to human and animal health and to vegetal production" (WHO, 2005). "LCT has been found to accumulate in biological membranes leading to oxidative damage by altering antioxidant systems and increasing lipid peroxidation (LPO) in mammals" [4].

"Fish is highly nutritious, easily digestible and a much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by water pollution" [5]. "The African cat fish, *Clarias gariepinus* is a remarkable and fascinating species, as it is extremely hardy and can withstand adverse environmental conditions and habitat instability. It is hardy and does not easily succumb to disease. It is one of the richest source of animal protein to man. Growth of this fish under natural condition is very fast as they can feed on all types of biowastes. *Claries gariepinus* is useful in biowaste management" [6]. It recycles different types of biowastes such as animal wastes poultry, butcher and fish wastes, and plant protein into fish protein. The fish has predatory, cannibalistic and the voracious feeding habit. It can efficiently assimilate a wide variety of animal and plant proteins and this has made most fish farmers to culture them in man-made inland freshwater bodies. Also, the fast growth rate and relatively high market price of this fish has lured many farmers into its culturing. Drained water from fish ponds can be used to irrigate vegetable crops. Fish and aquatic animals are exposed to pesticides in three primary ways. Dermal, direct absorption through the skin by swimming in pesticide-contaminated waters, Inhalation, by direct uptake of pesticides through the gills during respiration, and orally, by drinking pesticides - contaminated water or feeding on pesticide –contaminated prey. There are some secondary causes that cause the exposure of fish and aquatic animals to pesticides and eventually lead to toxicity. Through the consumption of another animal that has been poisoned by a pesticide. The exposure of fish and other aquatic life to pesticides may be a more widespread problem than most people realize. Most pesticide-related fish kills go unreported and, in documented cases, the number of fish killed is often underestimated. Scavengers quickly remove the bodies from the site of murder. Dying and stressed fish may hide in dense cover or leave the area completely.

"Bioaccumulation of toxic compounds in fish together with environmental stress can invoke the production of excess ROS commonly known

as free radicals- such as superoxide anion (O^{-2}), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH^-) can elicit physiological alterations, oxidative dysfunction such as lipid peroxidation" [7,8]. "Different cellular and extracellular components such as the nucleic acids are exposed to high risk of damages by these free radicals causing many degenerative and carcinogenic diseases. Many aquatic organisms show an ability to live in contaminated regions, principally due to their inducible defence mechanisms that allow detoxification, excretion, antioxidant protection, and stress response" (Bard, 2000).

"Fish antioxidant responses are very sensitive to environmental contamination and frequently used in aquatic environmental health monitoring" (Sturve et al. 2008). "However, when ROS generation exceeds the capacity of the cellular antioxidants, it will cause oxidative stress and oxidative damages. Presence of toxic substances in aquatic environment can be detected using fish as bioindicators owning to the fact that these substances bioaccumulate in them for a prolonged period of time. Herbicides are indiscriminately used with little or no regulation, and they persist in the environment for a long time. They are applied with the sole aim of controlling weeds, but they end up in aquatic environments thereby affecting non-target organisms including fish. They may lead to fish kill, affect fish behaviour, feeding, growth and ultimately reduce fish productivity" [9]. "Toxicity tests conducted at levels of lambda-cyhalothrin residues measured in water or sediment indicated potential for effects on aquatic organisms including fish and amphipods" [10-14] (Gu et al. 2007; Lawler et al. 2007; Van Wijngaarden et al. 2005; Wang et al., 2007; Weston et al., 2004). Concerns have therefore been raised about the widespread use of lambda. The aim of this study was to determine the impact of Lambda cyhalothrin on *Clarias gariepinus.*

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at Heldin's Fisheries Unit, Old Airport Road Thinkers Corner, Emene, Enugu State. Experiments were conducted to determine the impact of lambda cyhalothrin on African catfish *C. gariepinus* respectively. The agro pesticide Lambda cyhalothrin was used in the experiment. A total of one hundred and forty juveniles of *C. gariepinus* (mean weight 27 ± 0.07 g; mean length 13.48 ±1.01cm) were used for the experiment. The fishes were exposed to both acute toxicity test for 96 hrs and sub-lethal concentration of the pesticide for 15 days at 5days interval.

2.2 Collection and Acclimation of the Experimental Fish

All the fishes were obtained from Rojenny Tourist Game Village Idemmiri Local Government Area of Anambra State through the help of local fish farmers. They were transported to the Laboratory using 50litre gallon. The fishes were transferred into different fiber reinforced plastic (FRP) tanks, containing 15l of de-chlorinated tap water. Aeration was provided to all the containers round the clock with the help of aerator in order to maintain dissolved oxygen contents. This was made possible by providing the tanks with air stones and regulator valves to control the air pressure uniformly to all the tanks. The fishes were acclimated for two weeks before the commencement of the experiment. During the acclimation and throughout the exposure period, the fish were fed at 2% body weight with commercial fish diet (Coppens Fish feed for aquaculture by .5700 Am Helmond, Holland) with active ingredients of crude fibre 42%, crude fat 13%, crude fiber 2.8%, crude ash 6.6%, phosphorus 0.85%, sodium 0.2% and calcium 1.2%.

2.3 Experimental Design

Completely randomized design (CRD) was used for the experiment. One hundred and eighty fishes were distributed into eighteen plastic aquaria. Each treatment was replicated six times with 10 fish per container. Fishes were exposed to different sub lethal concentrations of the pesticides as treatments. The experiment was replicated in triplicates with the exception of the control. The different concentrations were measured and introduced into experimental containers containing 10litres of tap water. The mixture was allowed to stand for 30 minutes before introducing the fish to be tested.

2.4 Experimental set-up for Acute Toxicity Test (Range Finding Test)

The acute toxicity test of the pesticide to *C. gariepinus* was carried out according to Environmental Protection Agency (EPA) (2002) and United Nation Environmental programme (UNEP) (1989) in a static renewal system by

using 15L capacity plastic tanks. Five experimental concentrations of each of the pesticides were prepared for each of the experiments. The five concentrations of the pesticide were prepared from the original solution using the formula described by Solbe (1995). The concentrations of the trial test were prepared by pipetting different volumes of the original concentration of the pesticide into 10 L of water in five static tanks at a time to make five different solutions. In the experiment, the concentrations of Lambda cyhalothrin used were 1.25, 1.50, 3.00, 6.00, and 12.00 mg l^{-1}. Each concentration was prepared in replicate and used for stocking of ten fish. One group was exposed to only de-chlorinated tap water which served as control. Feed was not offered to the fish 48 hours before and during 96 hrs of test period. The physico-chemical parameters of the test water were analyzed using standard methods APHA [15]. The research was conducted in an indoor experimental outfit. The ethical guidelines for the Animal of Ministry of Agriculture, Enugu state were strictly adhered to. In order to avoid fouling the experimental media, the containers were checked daily, while the dead fishes were recorded and removed using scoop net. The water quality and appropriate concentrations of the pesticide were maintained by renewing the test water and the toxicant daily. The behavioral changes in the exposed fish and control were also observed. Finney's probit analysis method [16] was followed to determine the 96 hLC_{50} of the pesticides on the exposed fish.

2.5 Long-term Exposure to Sub-lethal Concentrations of the Agro Pesticides

The study was conducted in a static renewal system after the acute toxicity test. Each of the experiment was conducted by exposing the fish to different LC_{50} of the various pesticides at 96hrsThe concentrations of these pesticides were prepared in arithmetic series and were not sufficient to cause immediate mortality of the experimental fish. The concentrations of Lambda cyhalothrin used were 0.25, 0.50 and 1.00 mg l^{-1}. A total of one hundred and forty fishes from the acclimatized group were distributed randomly to the container, 10 fish per container. The fishes were fed twice daily at 2% total body weight at 9.00 and 16.00hrs with commercial fish diet, having 30% crude protein. Fifty per cent of the exposed solution was renewed every other day to maintain the water quality of the test media and normal concentration of the pesticides. Aeration was provided to all the containers round the clock with the help of aerator in order to maintain dissolved oxygen contents. This was made possible by providing air stones and regulator valves to control the air pressure uniformly to all the containers. The experimental containers were cleaned daily. The test fish along with the controls were sampled on days 5, 10 and 15 to determine the toxic effects of the pesticides on the exposed fish. On each sampling day, two fish from each concentration were used for the analysis of the antioxidant parameters.

2.6 Physico-chemical Parameters

Water quality parameters such as temperature, pH, and dissolved oxygen were recorded during the experimental period.

2.6.1 Temperature, pH and CO_2

These parameters were measured with a hand-held Hanna Combo instrument (HI 98129). The pH and temperature were measured by setting the pH mode on the instrument. The probe was submerged in the water for about one minute and the reading taken when the stability symbol on the top left corner of the LCD disappeared. The pH value was displayed on the primary LCD of the instrument while the secondary LCD displayed the temperature. The probe was submerged in water and the readings were taken as in the case of pH above. The primary LCD showed readings for both pH and CO_2 while the secondary LCD displayed the temperature of the sample.

2.6.2 Dissolved oxygen (DO)

Dissolved oxygen of the diluting water was measured according to the Winkler's method described by APHA [15]. This is described in the steps below:

- Water sample was poured into a 300ml biological oxygen demand (BOD) bottle. 2ml $MnSO_4$ solution was added followed by the addition of 2ml alkali-iodide azide reagent and the bottle stoppered with care to exclude air bubbles
- The sample was then mixed gently by inverting the bottle a number of times until a clear supernatant was obtained
- The sample was then allowed to settle for two minutes followed by the addition of 2ml H_2SO_4, allowing the acid to run down the neck of the bottle

- The bottle was stoppered again and gently inverted until dissolution was complete
- 100ml of the prepared solution was poured into a conical flask and titrated with 0.0125N $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow colour
- 3ml of freshly prepared starch solution was added, making the solution become blue in colour

Titration was continued by adding the thiosulphate drop-wise until the blue colour disappeared. The amount of thiosulphate used in the titration represented the amount of dissolved oxygen in the sample.

2.6.3 Alkalinity

Total alkalinity was determined titrimetrically as described by APHA (2012) using the following reagents: 0.1 N tetraoxosulphate (IV) acid and methyl orange indicator.

Procedure: One hundred millilitres (100 ml) of water was put in a conical flask and 2-1 drops of methyl orange indicator were added. Then it was titrated with 0.1 NH_2SO_4 until the yellow colour changed to orange indicating the end point. The total alkalinity (mg/l) was calculated from the following formula:

$$\text{Alkalinity (mgl/l)} = \frac{\text{ml x N x 50,000}}{V}$$

N = Normality of titrant
V = Volume of water sample (100 ml)
ml= Volume of titrant used.

2.7 Determination of Biochemical Indices

2.7.1 Total protein

Total protein was determined using Folin phenol reaction method as described by Lowry et al. 1951. 0.05ml of sample.

2.7.2 Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

ALT and AST in the liver and muscle of tissue homogenates were determined according to Wooten (1964). A substrate for ALT was prepared by dissolving $2x10^{-3}$ M α-ketoglutarate and 0.2 M DL-alanine in phosphate buffer of pH 7.7. The substrate for AST was prepared in the same manner but replacing DL-aspartate in place of DL-alanine. A mixture of 0.5 ml substrate and 0.1 ml tissue homogenate (enzyme) was incubated at 37^{o}C for 1 h. After, incubation period of 20 min with 0.5 ml of $2.5x10^{-4}$ M 2,4-dinitrophenyl hydrazine (DNPH) in 1 N HCl, 5 ml of 0.4 N NaOH was added to stop the reaction. Sample control was established by adding the enzyme only after the 1 h incubation time. A standard was prepared using 0.1 ml of $2x10^{-3}$ M sodium pyruvate in 0.5 ml 2,4-DNPH and incubated for 20 min. Absorbance reading at 540 nm was recorded for all the samples in triplicates. Enzyme specific activity was expressed in micromoles of pyruvate formed per minute per milligram protein.

2.7.3 Alkaline phosphatase

Liver alkaline phosphatases (ALP) were determined according to methods of Essey et al. [17]. Tissue homogenate (0.1 ml) was added into 0.2 ml, 0.2 M bicarbonate buffer (pH 9.5) with 0.1 ml, 0.1 M magnesium chloride solution in test tubes. After the addition of 0.5 ml distilled water and 0.1 ml 0.1M para-nitrophenyl phosphate (PNPP) substrate, the mixture was incubated at 37 ^{o}C for 15 min. Finally, 1 ml of 0.1 N NaOH was added to stop the reaction and absorbance was taken at 410 nm in a spectrophotometer. A sample control was prepared by the same method but, without the enzyme (tissue homogenate). A standard was also prepared using 0.1 ml, 100 µM para-nitrophenol phosphate (PNP) in place of the enzyme and without the substrate. Each sample was carried out in triplicates.

2.7.4 Acid phosphatase

The determination of same procedure was followed for the determination of ALP except the mixture of 0.2 M sodium acetate and acetic acid (pH 5) used as the buffer.

2.7.5 Statistical analysis

Data obtained were analysed, using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in test concentrations and control were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests to determine level of significance at 5% probability level. Results were expressed as mean ± standard deviation.

3. RESULTS

3.1 Effects of Lambda Cyhalothrin on Behavioural Response and Mortality of *Clarias gariepinus*

The fishes exposed to an acute concentration of Lambda cyhalothrin for 96 hrs, showed to some varying degrees of behavioural disorder before death. The behavioural irregularities displayed by the exposed fish increased with increasing concentrations of the Lambda cyhalothrin thus, exhibiting a positive correlation with the concentration.

At the onset of the experiment (12-24 hours post exposure), behavioural changes in fishes were rather rapid (Table 1). There was an immediate burst of activity (hyperactivity) in all the toxicant-exposed groups. This was characterized by abnormal or agitated/erratic swimming, colliding and hitting of tails against wall of the aquarium, sudden or quick movements as well as general restlessness. With progression of exposure time (48-72 hours post exposure), activity of fish in the exposed groups decreased (hyperactivity). There was air gulping (rapid opercular movement), loss of balance or equilibrium characterized by fish swimming backwards or in circles, free fall, as well as vertical positioning. Fish exhibited startle or panic responses to stimulus with sudden darts of energy and holding out their pelvic and pectoral fins. At the time of quiescence, fish exhibited highly reduced activity during which they remained vertically still with much reduced faint and irregular opercular beats. This period was followed by death. However, no abnormal changes were observed in the control experiment throughout the exposure duration.

Table 1. Behavioural response of *Clarias gariepinus* juvenile exposed to acute concentrations of Lambda cyhalothrin

Behavioural changes	Concentration (mgL-1)	Exposure duration			
		24 hrs	48 hrs	72 hrs	96 hrs
Air Gulping	Control	-	-	-	-
	0.25	+	+	+	
	0.50	+	++	+	++
	1.00	++	+	+	+++
Hyperactivity	Control	-	-	-	-
	0.25	-	++	+	+
	0.50	-	+++	++	+++
	1.00	+++	+++	+	+++
Erratic movement	Control	-	-	-	-
	0.25	+	+	+	+
	0.50	++	++	+	++
	1.00	++	++	+	+++
Skin Discoloration	Control	-	-	-	-
	0.25	++	+	++	+
	0.50	++	+	+	++
	1.00	++	++	+	+
	Control	-	-	-	-
Jerky movements					
	0.25	+	+	+	+
	0.50	+	++	+	+
	1.00	++	+	-	+++
	Control				
Equilibrium Status		-	-	-	-
	0.25	+	+	++	+
	0.50	+	++	+	+
	1.00	++	++	+	+++

Keys: *None -; Moderate ++; Strong +++; Mild +*

3.2 Physicochemical Parameters after Exposure to Lambda Cyhalothrin

Mean values of the water quality assay of both acute and long-term recorded during the exposure of *C. gariepinus* to Lambda cyhalothrin are presented in Table 2. The result showed that dissolved oxygen ranged from5.5 to 7.50 mgl^{-1}, temperature 27.50-28.00°C, pH 7.7-8.9 alkalinity 15.00-18.20 mg l^{-1}, while free carbon dioxide ranged from 4.2 -4.32 mg L^{-1} for acute test. In long term sub-lethal concentration test, dissolved oxygen ranged from 6.8 to 8.50 mg l^{-1}, temperature 27.50-28.00 °C, pH 7.8-9.1 alkalinity 18.00-22.20 mg l^{-1}, while free carbon dioxide ranged from 4.68-4.98 mgl^{-1}. Statistical analysis indicated that there was no significant difference ($P > 0.05$) in water quality parameters between the exposed tanks and the control.

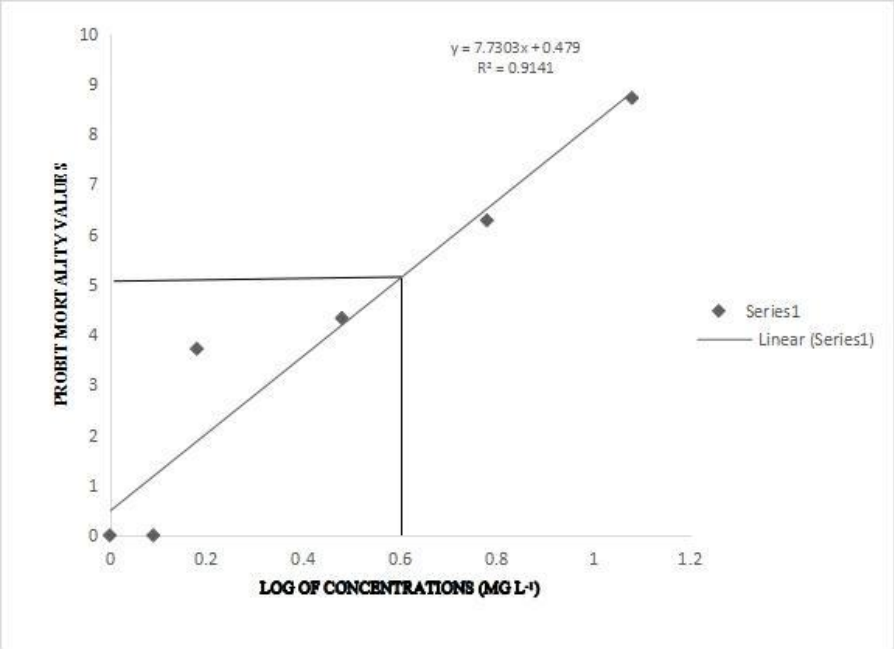

Fig. 1. Logarithmic concentration–probit line for determination of 96 hrs LC_{50} of Lambda cyhalothrin to *C. gariepinus*

Table 2. Mean values of physico-chemical parameter recorded during the exposure period

Parameter	Control		Acute test		Long-term exposure	
	Mean	Range	Mean	Range	Mean	Range
DO (mg L^{-1})	7.00	6.1-7.9	6.5	5.5-7.50	6.85	6.0-7.9
Temp (ºC)	27	27.0	26.25	27.5-28	28.25	27.5-29
pH	8.50	7.8-9.2	8.30	7.7-8.90	8.45	7.8-9.1
Alkalinity.(mgL^{-1})	17.85	16.2-19.5	17.1	16-18.20	17.75	16.0-19.5
CO_2 (mg L^{-1})	4.20	4.1-4.30	4.29	4.25-4.32	4.23	4.20-4.25

Table 3. Mean mortality and Probit values of *Clarias gariepinus* exposed to various concentrations of Lambda cyhalothrin

Lambda cyhalothrin (mgL^{-1})	Log concentration	Mean mortality (%)	Probit values
0.00	0.00	0	0
1.25	0.09	0	0
1.50	0.18	10	3.72
3.00	0.48	25	4.33
6.00	0.78	90	6.28
12.00	1.08	100	8.72

At 12.00 and 1.25 mgL^{-1} of Lambda cyhalothrin, 100 and 10% mortalities respectively, were observed in exposed fish while no mortality was recorded in the control group (Table 4).The 96 hrs LC_{50} of Lambda cyhalothrin to the exposed fish was found to be 3.98 mg L^{-1}.

3.3 Effect of Sub Lethal Concentration Lambda cyhalothrin on the Biochemical Parameters of *Clarias gariepinus*

As presented in (Table 4), there was significant difference (P<0.5) in the biochemical parameters analysed. There was increase in the mean values of Albumin (Album), aspartate amino transfarase (AST), alanine amino transaferase (ALT), Alkaline Phosphate (ALP). While a significant decrease was recorded in Creatinin . The highest value of Album recorded was 8.50±0.12 while the lowest values as 2.29±0.15. The highest and lowest value recorded for AST was 41.87±0.02 and 36.52±0.11 respectively. The highest and lowest values of ALT were 27.85 ± 0.2 and 26.09±0.05 respectively. ALP values peaked at 96.33±0.22 and the lowest values was 42.21±1.03. Total Protein was recorded to reduce significantly (P<0.5) as the number of days and concentrations reduced. The highest and lowest values of Total protein were 9.40±2.04 and 2.32±0.18.

Table 4. Biochemical parameter of *Clarias garipienus* juvenile to sub-lethal concentrations of Lambda cyhalothrin

Parameter	Propanil	Exposure duration (days)		
	Concentrations (mg/L^{-1})	**5**	**10**	**15**
Album	Control	2.48 ± 0.12^{1a}	3.64 ± 0.13^{1b}	3.43 ± 0.13^{2a}
	0.49	3.64 ± 0.12^{2c}	3.29 ± 0.15^{2c}	4.70 ± 0.11^{2b}
	0.98	3.24 ± 0.12^{2b}	6.76 ± 0.11^{1b}	4.80 ± 0.13^{1a}
	1.96	3.33 ± 0.06^{2b}	8.50 ± 0.12^{1b}	4.40 ± 0.11^{1a}
Creatinine	Control	4.34 ± 0.01^{1a}	6.34 ± 0.01^{2a}	6.35 ± 0.01^{2a}
	0.49	5.33 ± 0.01^{1c}	6.32 ± 0.02^{2c}	6.30 ± 0.02^{2b}
	0.98	5.30 ± 0.01^{1b}	7.27 ± 0.02^{2b}	7.22 ± 0.01^{1a}
	1.96	6.28 ± 0.01^{1c}	7.22 ± 0.04^{1b}	6.18 ± 0.04^{1a}
AST	Control	47.68 ± 0.12^{2a}	40.82 ± 0.12^{2a}	38.68 ± 0.05^{2a}
	0.49	46.45 ± 0.13^{1c}	62.44 ± 0.11^{2c}	38.30 ± 0.04^{2b}
	0.98	51.87 ± 0.11^{1c}	84.30 ± 0.09^{2c}	36.11 ± 0.02^{2b}
	1.96	61.22 ± 0.12^{1d}	83.10 ± 0.07^{1c}	40.30 ± 0.03^{1b}
ALT	Control	42.17 ± 1.07^{1a}	24.18 ± 0.12^{1a}	28.19 ± 1.02^{1a}
	0.49	40.30 ± 1.11^{1b}	32.21 ± 0.23^{1a}	30.21 ± 1.03^{1a}
	0.98	42.31 ± 1.08^{2a}	62.32 ± 0.18^{2a}	30.33 ± 1.03^{1b}
	1.96	49.39 ± 1.09^{4b}	61.33 ± 0.22^{2a}	35.40 ± 2.04^{2c}
ALP	Control	58.17 ± 1.07^{1a}	60.18 ± 0.12^{1a}	48.19 ± 1.02^{1a}
	0.49	66.30 ± 1.11^{1b}	81.21 ± 0.23^{1a}	40.21 ± 1.03^{1a}
	0.98	59.31 ± 1.08^{1a}	86.32 ± 0.18^{2a}	63.33 ± 1.03^{1b}
	1.96	59.39 ± 1.09^{4b}	95.33 ± 0.22^{2a}	59.40 ± 2.04^{2c}
Billi	Control	0.2 ± 0.12^{1a}	0.18 ± 0.12^{1a}	0.24 ± 0.12^{1a}
	1.25	0.52 ± 0.11^{3b}	0.28 ± 0.11^{2b}	0.18 ± 0.11^{1b}
	1.5	0.46 ± 0.11^{2a}	0.26 ± 0.11^{1b}	0.19 ± 0.11^{1b}
	1.75	0.21 ± 0.001^{1a}	0.18 ± 0.001^{2c}	0.21 ± 0.001^{2c}
T. Protein	Control	$6.6.17\pm1.07^{1a}$	6.18 ± 0.12^{1a}	9.19 ± 1.02^{1a}
	0.49	6.30 ± 1.11^{1b}	5.81 ± 0.23^{1a}	8.21 ± 1.03^{1a}
	0.98	6.31 ± 1.08^{2a}	3.32 ± 0.18^{1a}	8.33 ± 1.03^{1b}
	1.96	7.39 ± 1.09^{4b}	3.33 ± 0.22^{1a}	8.40 ± 2.04^{2c}

Mean with different alphabetic letters (a,b and c) show significance difference (p<0.05) among Lambda cyhalothrin concentrations within the rows while different numeric(1,2 and 3) superscripts indicate significant difference among durations of exposure within the horizontal as determined by Duncan's multiple Range

4. DISCUSSION

The physico-chemical parameters of the test water measured during both acute and sub-lethal toxicity bioassay were within suitable ranges for the survival and normal growth of *C. gariepinus*. Hence changes in fish behaviour and subsequently death could not have arisen from poor water quality of the test water. On the optimum pH scale for fish growth developed by Badiru [18], the range of pH for this study (7.8-9.26) corresponds to the desirable range (6.5-9) for fish production. However, dissolved oxygen range for this study (6.1-9.5 mg/L) spans the range for slow growth following long term exposure (15mg/L) of the dissolved oxygen scale for warm water fishes by Badiru [18]. Similarly, the temperature range for this study (16.4-20°C) is within the normal range of temperature in the tropics to which fish are adapted (22-35°C) as reported by Howerton [19].

Changes in behaviour observed in this study are similar to those reported by several authors. Hyperactivity of fish in exposed groups during 12-24 hours could be attributed to an attempt to escape the toxic environment. Hyperactivity of fish on introduction to an unfavourable environment has been suggested as the primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities. Ramesh et al. [20] reported similar behavioural responses of common carp to atrazine exposure which include increased opercula movement, mucous secretion, jerky movement, floating on the sides and hypersensitivity showing violent erratic and fast swimming, and opined that the abnormal behaviour of the fish indicates the toxic effect of Lambda cyhalothrin on the central nerves system (CNS) and cardiovascular system. Mekkawy et al. [1] also reported "hyperactivity in *C. gariepinus* exposed to atrazine which was characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid pectoral fins and opercula movements, reduction in the feeding activity, fins haemorrhage and loss of some skin parts". In the course of metribuzin poisoning in rainbow trout, Velisek et al. [21] reported similar clinical symptoms such as accelerated respiration, loss of movement coordination, fish lying on their flanks and moving in this position. Swollen abdomen and discolouration of the skin were also observed in fish exposed to the toxicant. This is attributable to necrotic damage to the gut of fish and suggests that toxicity of both pesticides is not restricted only to the outside. Annune et al. [22] and Olusegun [23] reported similar findings. Nwani et al. [24] also reported "skin discolouration in *Tilapia zilli* exposed to the chloroacetanilide herbicide butachlor". Ikele et al. [25] similarly observed that the "*C. gariepinus* normal darkly pigmentation in the dorsal and lateral parts was changed to very light pigmentation when exposed to diethyl phthalate. Generally, it is argued that behavioural studies gives a direct picture of response of the fish to pesticides and related chemicals and the behavioural activity as well as morphological responses of organisms represents the final integrated result of a diversified biochemical and physiological processes".

The present study demonstrated that *C. gariepinus* exposed to lambda-cyhalothrin showed concentration and duration dependent on significant increase ($P< 0.05$) in radical activities.. "Determinations of ALT and ALP enzymes in blood plasma are a sensitive indicator of cellular damage, organ malfunction and water pollution" [26]. In this study, ALT and ALP activities were elevated in tissues indicating the disorder in Kreb's cycle caused by the pesticides; Lambda cyhalothrin. Damage in hepatic cells may have been responsible for the significant increases in the enzymes. The elevations of ALT and ALP activity indicated that the fish tried to mitigate the pesticide induced stress. The elevations were due to damage in hepatic cells (necrosis, apoptosis or both). Increase in serum ALP activity suggests leakage of the enzyme into the blood as a result of tissue necrosis. High serum ALP as in the present study was also reported in the teleost *Clarias batrachus* treated with endosulphan and kelthane, suggesting cell necrosis and an increase in lysosomal mobilization [27]. "The accumulation and binding of both pesticides in the tissues of the exposed fish could induce stressful conditions and subsequently results in the elevation of the transamination pathway to counter the resultant energy crisis" [28]. "The significant increase in serum ALT, AST and ALP level was testified in *C. carpio* treated with profenofos" [29]. Similar findings were also reported in serum of *Oreochromis niloticus* exposed to endosulfan, *C. carpio* exposed to lufenuron, *Oreochromis niloticus* exposed to deltamethrin [30] (Dawood et al. 2020a; Hussein et al. 2019). Increase in ACP activity in the treated fish could be due to the destruction of lysosomal membrane which resulted in the release of the enzyme to the tissues. Akinpelu et

al. (2013), Rahman et al. [29] and Singh et al. [31], reported increase in ACP activities in fish exposed to toxins [32,33].

5. CONCLUSION

From the study, Lambda cyhalothrin and Propanil were found to be moderately toxic to *C. gariepinus* with an LC_{50} value of 3.98 mg L^{-1} and 7.94 mg L^{-1} respectively. Changes in behaviour and morphology observed in the species included hyperactivity, startled responses, rapid opercular movement, loss of balance, skin discolouration, holding out pectoral and pelvic fins, swollen abdomens, mucus secretion and a period of quiescence prior to death. Exposure of the fish to acute and sub-lethal concentrations of the toxicants resulted in a number of significant changes in the oxidative, biochemical and haematology response of the species.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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